Supplementary information 1

Laser microirradiation and live-cell imaging. To combine laser microirradiation with an immediate image acquisition, the PALM UV-A pulse nitrogen laser (30 Hz, λ=337 nm; Palm Microlaser, Bernried; Germany) was mounted on the Axiovert 200 microscope (Zeiss) on a custom-designed granite plate. The UV laser beam was coupled directly to the epifluorescence path, and focused through a 40x C-Apochromat water-immersion objective (Zeiss) to yield a spot size of approximately 0.5 μm. The output of the UV laser was set to 50%, a minimal dose required to generate a clearly detectable DSB response strictly within the laser-exposed nuclear compartments in a BrdU-dependent manner (see Supplementary information 6). Operation of the UV laser was assisted by the PALMRobo-Software supplied by the manufacturer. The total time of a single cell exposure to laser beam did not exceed 1 second. Under these conditions we did not observe adverse morphological or cytotoxic effects for up to 48 hours. Immediately after microirradiation, the same field was subjected to a repeated image acquisition via the Zeiss LSM META unit integrated into the same Axiovert 200 microscope and operated by the LSM 510 software. The first images were consistently recorded around 5 - 6 s after DSB generation. The emission fingerprinting features of the META spectral detector allowed accurate separation of fluorophores with close emission spectra, thereby facilitating simultaneous evaluation of GFP- and YFP-tagged protein mobility under identical experimental conditions. Images were acquired in a 12-bit mode. The fluorescence intensities were placed within the linear range of the detector by setting the amplifier offset to zero, and the detector gain adjusted so that the peak intensities never

reached saturation levels. The residual noise of the detector never exceeded 5% of the specific fluorescence values.

The kinetics of the DNA damage-induced redistribution of the GFP(YFP)-tagged proteins was measured by recording the specific fluorescence within the microirradiated areas in 20-s intervals and for the total time period specified in Figure legends. The measured values were corrected for non-specific fluorescence bleaching during the repeated image acquisition and processed as follows. For comparison of Nbs1-2YFP and GFP-Mdc1 recruitment to DSB, the relative fluorescence (RF) was calculated as $RF_{(t)} = (I_t – I_{preIR})/ I_{max} – I_{preIR})$, where I_{preIR} is the fluorescence intensity measured in the laser exposed nuclear areas immediately before microirradiation, and I_{max} represents the maximum fluorescence signal after saturated GFP(YFP)-tagged protein accumulation in the DSB regions. The redistribution of fluorescence was fitted to a mathematical model for a first order step response: $RF_{(t)} = 1\text{-}\exp(-t/\tau)$. Data from individual cells were treated as individual experiments and fitted to this one-parameter model with a least-squares method. Statistical analysis of the calculated recruitment time constant (τ) as well as visual inspection of the fits provided the basis for comparison of the redistribution kinetics of Nbs1 vs. Mdc1.

To compare DSB binding of Nbs1 in siRNA-treated cells with that of various Nbs1 mutants, we determined the fold of increase of specific GFP-associated fluorescence (RF_{fold}) within the microirradiated areas according to the following formula: $RF_{fold(t)} = I_t/I_{preIR}$, where I_{preIR} is the fluorescence intensity measured in the laser-exposed areas immediately before irradiation.

Unless stated otherwise in Figure legends (see Fig. 1B, where the mathematical modelling of Mdc1 and/or Nbs1 recruitment to DSBs is exemplified by the data from one cell), the plotted data throughout the manuscript integrate measurements from 10 independent, randomly chosen cells, and all kinetic measurements in this study (including FRAP analyses; see below) were reproduced in at least two independent cell lines expressing the indicated GFP/YFP-tagged protein.

Photobleaching assays. For FRAP analysis, a rectangular scanning area was placed over the DSB-containing nuclear stripes or to the neighbouring undamaged nucleoplasm as indicated in figure legends. Following a series of 10 pre-bleach scans, a smaller region of a constant size (0.5 μm^2) was exposed to a single 0.2-s bleach pulse by the 488- or 514-nm argon laser set to 25% output and 100% transmission. This bleach pulse was followed by 76-ms interval image acquisitions of the entire scanning area (90 in total) with the laser transmission attenuated to 1% and the pinhole set to 2.5 AU (see also the schematic representation of the FRAP assays in Fig. 2).

To display the FRAP curves, the relative fluorescence (RF) was calculated as $RF_{(t)} = I_t/I_{pre}$, where I_{pre} is the highest pre-bleach value for each measurement. Such a normalization procedure allows to visually appreciate the extent of immobile fractions of the studied proteins and to see the efficiency of fluorescence loss during the bleach pulse (in addition to the calculated residence times, see below, these parameters further illustrate the relative mobility of proteins within the DSB-containing nuclear compartments).

To determine the residence time of Nbs1 and Mdc1 at the sites of DNA damage, the relative fluorescence (RF) was calculated and normalized as follows: $RF_{(t)} = (I_t–I_0)/$

(I_{post}–I_0), where I_0 is the fluorescence intensity immediately after bleaching, and I_{post} represents the fluorescence intensity after complete recovery. This normalized recovery of fluorescence was recognized to constitute a cumulative distribution curve [$F_{(t)}$] of residence times for individual protein molecules at damaged DNA. The data was successfully fitted with a least-squares method to a standard model for such a curve: $F_{(t)} = 1-\exp(-t/t_m)$. The single parameter of this model, the mean residence time (t_m), was used for comparison of the two proteins. The t_m constitutes a representative residence time for all protein molecules at the site of damage, always corresponding to 63.2 % fluorescence recovery. For mathematical reasons, the standard deviation of t_m found from the above expression is always as large as the t_m itself.

To determine effective diffusion coefficient (D_{eff}) of Nbs1 and Mdc1 in undamaged nuclei, the FRAP conditions were adjusted according to (Essers et al., 2002). Briefly, a bleach/scanning region with a constant width of 2 μm was placed across the entire nuclear diameter, bleached for 300 ms, and the recovery was monitored at intervals of 115 ms for the period of 10 s. The settings for bleaching and the subsequent image acquisition were as described above. After correction for the non-specific fluorescence loss during the image acquisition, the relative fluorescence (RF) was determined as $RF_{(t)} = (I_t–I_0)/(I_{post}–I_0)$, where I_{post} is the fluorescence intensity measured after complete recovery and I_0 is the fluorescence intensity immediately after bleaching. D_{eff} was calculated by fitting the normalized data to the mathematical model described in (Essers et al. 2002). To avoid the rather large systematic error of the model, only the fluorescence recovery within the first second was used for calculation of D_{eff}.